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DuPont SHE Excellence Center

AR201-13125

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July 17, 2001

Christine Todd Whitman
Administrator
U.S. EPA
P.O. Box 1473
Merrifield, VA 22116

Attn: Chemical Right-to-Know Program

Dear Administrator Whitman:

E. I. du Pont de Nemours & Co., Inc. is pleased to submit the proposed test plan along with the robust summary for the chemical Glycolic acid, CAS# 79141. DuPont understands there will be a 120-day review period for the test plan and that all comments received by EPA will be forwarded to DuPont for consideration.

This submission includes one electronic copy in .pdf format.

Please feel free to contact me with any questions or concerns you might have concerning this submission at Edwin.L.Mongan-1@usa.dupont.com or 302-773-0910.

Sincerely

Edwin L. Mongan III
Manager, Environmental Stewardship

ELM
Enclosure

CC: Charles Auer – U.S. EPA

16-July-2001

AR201-13124A

ROBUST SUMMARY FOR 1,1-DIFLUOROETHANE

Summary

1,1 -Difluoroethane (HFC- 152a) is expected to exist solely in the vapor-phase in the ambient atmosphere. Vapor-phase HFC- 152a is degraded in the atmosphere by reaction with photochemically-produced hydroxyl radicals with an atmospheric half-life of about 472 days. The long atmospheric lifetime of this chemical suggests that some HFC-152a is expected to gradually diffuse into the stratosphere above the ozone layer where it will slowly degrade due to direct photolysis from UV-C radiation. In water, HFC-152a is not expected to adsorb to sediment or particulate matter and it is expected to volatilize rapidly from water surfaces. Estimated half-lives for a model river and model lake are 2 and 77 hours, respectively. Bioconcentration is expected to be low based upon an estimated BCF value of 2. Highly chlorinated/fluorinated compounds are not expected to biodegrade rapidly (Boethling et al., 1994), thus, biodegradation is not expected to be an important environmental fate process (HSDB, 2000). Since no test data are available, further evaluation is needed for confirmation.

HFC- 152a is a gas at room temperature and has low acute toxicity in the mammalian systems evaluated via inhalation. The inhalation studies were conducted using Good Laboratory Practices (GLP) and demonstrate that HFC-152a behaves similarly to other structurally similar fluorocarbons. In addition to the acute inhalation toxicity studies, a 2-year inhalation study with a 3-month interim sacrifice was conducted with HFC-152a. No significant toxicological effects were observed at the 3-month sacrifice and no histopathological or weight effects on reproductive organs of either male or female rats were observed in the 2-year bioassay. HFC-152a was not a developmental toxicant in a rat developmental toxicity study. HFC-152a was not mutagenic in the in *vitro* bacterial reverse mutation test (Ames test) in *Salmonella typhimurium* and *Escherichia coli* strains. However, HFC- 152a showed evidence of weak clastogenicity in an *in vitro* human lymphocyte chromosome aberration test. Further evaluation of the chromosome aberration potential using an *in vivo* micronucleus test produced negative results.

No ecotoxicological studies have been conducted with HFC- 152a and there is very little or no ecotoxicology data for similar non-chlorinated, fluorocarbon compounds. Modeling of several physical-chemical parameters, fate processes, and aquatic toxicity was conducted to help provide insight into the behavior in the environment and the aquatic toxicity of a homologous series of 4 fluorocarbon compounds (HFC-152a, HFC- 134a, HFC- 125, and hexafluoroethane).

Syracuse Research Corporation models for estimating physical-chemical properties and fate processes were used to estimate $\log_{10}$ Kow (Meylan and Howard, 1995), water solubility at 25°C (Meylan and Howard, 1996), Henry's Law Constant (Meylan and Howard, 1991), and ultimate biodegradation (Boethling et al., 1994). The dominant fate

process controlling distribution of these compounds in the environment is volatilization (Mackay et al., 1996).

Compound	$\log_{10}$ Kow (Estimated)	Water Solubility	Henry's Law Constant	Ultimate Degradation
HFC- 152a ($C_2H_4F_2$)	1.13	2671 mg/L	3. SE-O 1 atm-m^3/mole	Weeks
HFC-134a ($C_2H_2F_4$)	1.68	768 mg/L	1.5E00 atm-m^3/mole	Weeks-months
HFC-125 (C_2HF_5)	1.55	867 mg/L	3.1 EOO atm-m^3/mole	Weeks-months
Hexafluoroethane (C_2F_6)	2.15	223 mg/L	2.4E+01 atm-m^3/mole	Months

ECOSAR (Meylan and Howard, 1999) was used to predict the aquatic toxicity of the 4 fluorocarbon compounds to green algae, daphnids (planktonic freshwater crustaceans), and fish. ECOSAR predictions are based on actual toxicity test data for classes of compounds with similar modes of action, i.e., narcosis in the case of fluorocarbons. Predicted $\log_{10}$ Kow values were used as input for the ECOSAR model. To help gauge the sensitivity of the prediction to this parameter, ECOSAR predictions were made using 2 Kow values. The initial Kow value was based on the estimated value from the Syracuse Research Corporation model except for the measured value of 0.75 for HFC-152a (Jow and Hansch, 1995). The second Kow value was empirically selected to be approximately $\log_{10}$ 0.5 greater than the initial measured or estimated value.

Compound	$\log_{10}$ Kow	Algae, 96-hr EC_{50}	Daphnid, 48-hr EC_{50}	Fish, 96-hr LC_{50}
		(mg/L)	(mg/L)	(mg/L)
HFC-152a	**0.75***	**419**	**720**	**733**
($C_2H_4F_2$)	**1.5**	**91**	**150**	**145**
HFC-134a	1.5	140	231	223
($C_2H_2F_4$)	2.0	51	81	76
HFC-125	1.5	165	272	263
(C_2HF_5)	2.0	60	95	89
Hexafluoroethane	2.0	69	110	102
(C_2F_6)	2.5	25	38	35

* measured value

The only actual test data available are for HFC-134a. Results of aquatic testing of this compound with daphnids and fish indicated that the daphnid 48-hour EC_{50} was 980 mg/L and the 96-hour fish LC_{50} was 450 mg/L (Stewart and Thompson, 199 I ; Thompson,

1991). Based on the ECOSAR predictions, the actual toxicity test data, and the high Henry's Law Constant for these compounds, HFC-152a is unlikely to represent an unacceptable risk to aquatic organisms or wildlife.

Emissions from HFC- 152a manufacturing facilities are small and industrial hygiene monitoring data during manufacture and industrial use show exposure to be well under acceptable exposure limits. Though consumer exposure has not been measured directly, modeling based on measurement of similar uses shows consumer exposure to be minimal during intended uses. Due to its low toxicity and low exposure potential, HFC-152a does not represent an unacceptable risk to human health or the environment. Although many of the studies available for HFC- 152a were conducted prior to implementation of the OECD Guidelines, much of the data available are scientifically valid. While a specific guideline reproductive toxicity test was not conducted, histopathological evaluation and organ weight measurements were made on reproductive organs during the 2-year inhalation study.

References for the Summary:

Boethling, R. S. et al. (1994). Environ. Sci. Technol., 28:459-465 (BIOWIN Software available from Syracuse Research Corp., Environmental Science Center, Syracuse, NY 13210).

HSDB (2000). Hazardous Substance Data Bank (HSDB/5205).

Jow, P. and Hansch, C. (n.d.). Pomona College, unpublished analysis (cited in Hansch, C. and A. Leo (1995). Exploring QSAR Fundamentals and Applications in Chemistry and Biology, Amer. Chem. Soc., Washington, DC).

Mackay, D. et al. (1996). Environ. Toxicol. Chem., 15(9):1627-1637.

Meylan, W. M. and P. H. Howard (1991). Environ. Toxicol. Chem., 10:1283-1293 (HENRYWIN Software available from Syracuse Research Corp., Environmental Science Center, Syracuse, NY 13210).

Meylan, W. M. and P. H. Howard (1995). J. Pharm. Sci., 84:83-92.

Meylan, W. M. and P. H. Howard (1996). Environ. Toxicoi. Chem., 15:100-106.

Meylan, W. M. and P. H. Howard (1999). User's Guide for the ECOSAR Class Program, Version 0.993 (Mar 99), prepared for J. Vincent Nabholz and Gordon Cas, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC, prepared by Syracuse Research Corp., Environmental Science Center, Syracuse, NY 132 10 (submitted for publication).

Stewart, K. M. and R. S. Thompson (1991). ICI Group Environmental Laboratory Report No. BL3908/B, ICI, UK (cited in Berends, A. G. et al. (1999). Arch. Environ. Contam. Toxicol., 36(2): 146-15 1).

Thompson, R. S. (199 1). ICI Group Environmental Laboratory Report No. BL4035/B, ICI, UK (cited in Berends, A. G. et al. (1999). Arch. Environ. Contam. Toxicol., 36(2):146-151).

16-July-2001

TEST PLAN FOR 1,1-DIFLUOROETHANE

1,1-Difluoroethane (HFC-152a) CAS No. 75-37-6	Data Available	Data Acceptable	Testing Required
	Y/N	Y/N	Y/N
PHYSICAL/CHEMICAL CHARACTERISTICS			
Melting Point	Y	Y	N
Boiling Point	Y	Y	N
Vapor Pressure	Y	Y	N
Partition Coefficient	Y	Y	N
Water Solubility	Y	Y	N
ENVIRONMENTAL FATE			
Photodegradation	Y	Y	N
Stability in Water	Y	Y	N
Transport (Fugacity)	Y	Y	N
Biodegradation	N	N	Y
ECOTOXICITY			
Acute Toxicity to Fish	Y	Y	N
Acute Toxicity to Invertebrates	Y	Y	N
Acute Toxicity to Aquatic Plants	Y	Y	N
MAMMALIAN TOXICITY			
Acute Toxicity	Y	Y	N
Repeated Dose Toxicity	Y	Y	N
Developmental Toxicity	Y	Y	N
Reproductive Toxicity	Y	Y	N
Genetic Toxicity Gene Mutations	Y	Y	N
Genetic Toxicity Chromosomal Aberrations	Y	Y	N

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The studies listed below were selected to represent the best available study design and execution for these HPV toxicity endpoints. Other data of equal or lesser quality are not summarized, but are listed as additional references in this document.

1.0 Substance Information

CAS Number:	**75-37-6**
Chemical Name:	Ethane, 1,1-difluoro-
Structural Formula:	$F_2 - HC - CH_3$
Other Names:	HFC- 152a; Freon@ 152a; Ethylidene fluoride; Genetron® 152a; Algofrene 67; Dymel 152; Dymel 152a; F152A; FC152a; FKW 152a; HFA 152a; R 152a; Ethylene fluoride; Ethylidene difluoride; Genetron 100
Exposure Limits:	1000 ppm, 8-hour TWA: AIHA WEEL 1000 ppm, 8-hour TWA: DuPont Acceptable Exposure Limit (AEL)

2.0 Physical/Chemical Properties

2.1 Melting Point

Value:	**-117°C**
Decomposition:	No
Sublimation:	No Data
Method:	No Data
GLP:	Unknown
Reference:	Lewis, R. J., Sr. (1993). Hawley's Condensed Chemical Dictionary, 12th ed., p. 399, Van Nostrand Reinhold Co., New York.
Reliability:	Not assignable because limited study information was available.

Additional References for Melting Point: None Found.

2.2 Boiling Point

Value:	-24.7°C
Decomposition:	No Data
Pressure:	No Data
Method:	No Data
GLP:	Unknown

Reference:	Lewis, R. J., Sr. (1993). Hawlev's Condensed Chemical Dictionary, 12th ed., p. 397, Van Nostrand Reinhold Co., New York.
Reliability:	Not assignable because limited study information was available.

Additional Reference for Boiling Point:

DuPont Co. (1998). Material Safety Data Sheet No. DUO05704 (November 4).

2.3 Density

Value:	0.90 mg/mL
Temperature:	25°C
Method:	No Data
GLP:	Unknown
Results:	Determined for liquid density
Reference:	DuPont Co. (1998). Material Safety Data Sheet No. DUO01260 (November 4).
Reliability:	Not assignable because limited study information was available.

Additional Reference for Density:

Kirk-Othmer Encyclopedia of Chemical Technology (199 1 -present). Volume 1, p. 677, John Wiley and Sons, New York.

2.4 Vapor Pressure

Value:	4550 mm Hg
Temperature:	25°C
Decomposition:	No Data
Method:	No Data
GLP:	Unknown
Reference:	Dauber-t and Danner (1989). Physical and Thermodynamic Properties of Pure Chemicals Data Compilation, Taylor and Francis, Washington, DC.
Reliability:	Not assignable because limited study information was available.

Additional References for Vapor Pressure:

DuPont Co. (1998). Material Safety Data Sheet No. DUO05704 (November 4).

Aviado, D. M. and D. G. Smith (1975). Toxicology, 3:241-252.

2.5 Partition Coefficient (log Kow)

Value:	0.75
Temperature:	No Data
Method:	Measured
GLP:	Unknown
Reference:	Jow, P. and Hansch, C. (n.d.). Pomona College, unpublished analysis (cited in Hansch, C. and A. Leo (1995). Exploring OSAR Fundamentals and Applications in Chemistry and Biology, Amer. Chem. Soc., Washington, DC).
Reliability:	Not assignable because limited study information was available.

Additional Reference for Partition Coefficient (log Kow):

Meylan, W. M. and P. H. Howard (1995). J. Pharm. Sci., 84:83-92.

2.6 Water Solubility

Value:	2.8 g/L
Temperature:	25°C
pH/Pka:	No Data
Method:	No Data
GLP:	Unknown
Reference:	DuPont Co. (1998). Material Safety Data Sheet No. DUO01260 (November 4).
Reliability:	Not assignable because limited study information was available.

Additional Reference for Water Solubility:

Ruelle, P. and U. W. Kesselring (1997). Chemosphere, 34:275-298.

Meylan, W. M. and P. H. Howard (1996). Environ. Toxicol. Chem., 15:100-106.

2.7 Flash Point

Value:	< -50°C
Method:	Open Cup
GLP:	Unknown
Reference:	DuPont Co. (1998). Material Safety Data Sheet No. DUO01260 (November 4).
Reliability:	Not assignable because limited study information was available.

Additional References for Flash Point: None Found.

2.8 Flammability

Results:	3.7% - 18% (in air)
Method:	No Data
GLP:	Unknown
Reference:	Sax, N. I. and R. J. Lewis, Sr. (1987). Hawley's Condensed Chemical Dictionary, 11th ed., p. 397, Van Nostrand Reinhold Co., New York.
Reliability:	Not assignable because limited study information was available.

Additional Reference for Flammability:

DuPont Co. (1998). Material Safety Data Sheet No. DUO01260 (November 4).

3.0 Environmental Fate

3.1 Photodegradation

Concentration:	Not Applicable
Temperature:	Not Applicable
Direct Photolysis:	Not Applicable
Indirect Photolysis:	Not Applicable
Breakdown Products:	Not Applicable
Method:	According to a model of gas/particle partitioning of semivolatile organic compounds in the atmosphere (Bidleman, 1988), 1,1 -difluoroethane is expected to exist solely as a vapor in the ambient atmosphere. The rate constant for the vapor-phase reaction of 1,1 -difluoroethane with photochemically-produced hydroxyl radicals has been measured as 3.4×10^{-14} cm^3/molecule-sec (Atkinson, 1989). This corresponds to an atmospheric half-life of about 472 days at an atmospheric concentration of 5×10^5 hydroxyl radicals per cm^3 (Atkinson, 1989). The long atmospheric lifetime of this chemical suggests some 1,1 -difluoroethane is expected to gradually diffuse into the stratosphere above the ozone layer where it will slowly degrade due to direct photolysis from UV-radiation (Nimitz and Skaggs, 1992; SRC, n.d.). 1,1 -Difluoroethane is not expected to undergo hydrolysis or direct photolysis in the troposphere due to the lack of functional groups to hydrolyze or absorb UV light at environmentally significant wavelengths (SRC, n.d.).

GLP: Not Applicable
Reference: Atkinson, R. (1989). J. Phys. and Chem. Reference Data (HSDB/5205).

Nimitz, J. S. and S. R. Skaggs (1992). Environ Sci. Technol., 26:739-44 (HSDB/5205).

Bidleman, T. F. (1988). Environ. Sci. Technol., 22:361-367 (HSDB/5205).

SRC (Syracuse Research Corporation) (n.d.). (HSDB/5205).
Reliability: Estimated value based on accepted model.

Additional Reference for Photodegradation:

Data from this additional source support the study results summarized above. This study was not chosen for detailed summarization because the data were not substantially additive to the database.

Ko, M. K. W. and N. D. Sze (1997). Final Report on Modeling Studies to Assess the Environmental Effects of Alternative CFCs (TSCA Fiche OTS0558956).

3.2 Stability in Water

Concentration: Not Applicable
Half-life: Estimated half-lives for a model river (1 m deep, flowing 1 m/sec, wind velocity of 3 m/sec) and model lake (1 m deep, flowing 0.05 m/sec, wind velocity of 0.5 m/sec) are 2 and 77 hours, respectively (Lyman et al., 1990; SRC, n.d.). Based on a recommended classification scheme (Swann et al., 1983), an estimated Koc value of 60 (SRC, n.d.), determined from a measured log Kow of 0.75 (Jow and Hansch, n.d.) and a recommended regression-derived equation (Lyman et al., 1990), 1,1 -difluoroethane is not expected to adsorb to suspended solids and sediment in water (SRC, n.d.). 1,1-Difluoroethane is expected to volatilize rapidly from water surfaces (Lyman et al., 1990; SRC, n.d.) based on an estimated Henry's Law constant of 0.02 atm-m^3/mole (Daubert and Danner, 1989; Ruelle and Kesselring, 1997; SRC, n.d.).
% Hydrolyzed: Not Applicable
Method: Modeled, estimated
GLP: Not Applicable
Reference: Swann, R. L. et al. (1983). Res. Rev., 85:23 (HSDB/5205).

Jow P. and Hansch, C. (n.d.). Pomona College, unpublished analysis (cited in Hansch, C. and A. Leo (1995). Exploring QSAR Fundamentals and Applications in Chemistry and Biology, Amer. Chem. Soc., Washington, DC).

Lyman, W. J. et al. (1990). Handbook of Chemical Property Estimation Methods, pp. 4-9, 5-4, 5-10, 15-1 to 15-29, Amer. Chem. Soc., Washington, DC (HSDB/5205).

Daubert, T. E. and R. P. Danner (1989). Physical and Thermodynamic Properties of Pure Chemicals Data Compilation, Amer. Inst. Chem. Eng., Hemisphere Pub, Corp., New York (HSDB/5205).

Ruelle. P. and U. W. Kesselring (1997). Chemosphere, 34:275-98 (HSDB/5205).

SRC (Syracuse Research Corporation) (n.d.). (HSDB/5205).

Reliability: Estimated value based on accepted model.

Additional References for Stability in Water: None Found.

3.3 Transport (Fugacity):

Media:	Air, Water, Soil, Sediments
Distributions:	Air: 99.9% Water: 0.111% Soil: 0.01% Sediment: <0.01%
Adsorption Coefficient:	Not Applicable
Desorption:	Not Applicable
Volatility:	Not Applicable
Method:	Calculated according to Mackay, Level 111, Syracuse Research Corporation Epiwin Version 3.05 Emissions to Air (1000kg/hr) EPA Model Defaults.
	Data Used: Molecular Weight: 66.05 Henry's Law Constant: 0.0203 atm-m^3/mole (Henry database) Vapor Pressure: 3.86×10^3 (Mpbpwin program) Log Kow : 0.75 (Jow and Hansch, n.d.) Soil Koc : 35 (Pckocwin program)
GLP:	No

Reference: Syracuse Research Corporation EPIWIN v3.05 contains a Level 111 fugacity model. The methodology and programming approach was developed by Dr. Donald Mackay and co-workers which is detailed in:

Mackay, D. (199 1). Multimedia Environmental Models; The Fugacity Approach, pp 67-l 83, Lewis Publishers, CRC Press.

Mackay, D. et al. (1996). Environ. Toxicol. Chem., 15(9):1618-1626.

Mackay, D. et al. (1996). Environ. Toxicol. Chem., 15(9): **1627-1637.**

Jow, P. and Hansch, C. (n.d.). Pomona College, unpublished analysis (cited in Hansch, C. and A. Leo (1995). Exploring QSAR Fundamentals and Applications in Chemistry and Biology, Amer. Chem. Soc., Washington, DC).

Reliability: Estimated value based on accepted model.

Additional References for Transport (Fugacity): None Found.

3.4 Biodegradation: No Data.

3.5 Bioconcentration

Value: BCF 2

Method: BCF was determined using a measured log Kow of 0.75 (Jow and Hansch, n.d.) and a recommended regression-derived equation (Lyman et al., 1990). According to a classification scheme (Franke et al., 1994), the estimated BCF value of 2 suggests that bioconcentration in aquatic organisms is low (SRC, n.d.).

GLP: Not Applicable

Reference: Jow, P. and Hansch, C. (n.d.). Pomona College, unpublished analysis (cited in Hansch, C. and A. Leo (1995). Exploring QSAR Fundamentals and Applications in Chemistry and Biology, Amer. Chem. Soc., Washington, DC).

Lyman, W. J. et al. (1990). Handbook of Chemical Property Estimation Methods, pp. 5-4, 5-10, Amer. Chem. Soc., Washington, DC (HSDB/5205).

Franke, C. et al. (1994). Chemosphere, 29:1501-14 (HSDB/5205).

SRC (Syracuse Research Corporation) (n.d.). (HSDB/5205).
Reliability: Estimated value based on accepted model.

Additional References for Bioconcentration: None Found.

4.0 Ecotoxicity

4.1 Acute Toxicity to Fish

Type:	**96-hour LC_{50}**
Species:	Fish
Value:	**733** mg/L (log_{10} Kow of 0.75), 145 mg/L (log_{10} Kow of 1.5)
Method:	Modeled
GLP:	Not Applicable
Test Substance:	HFC- 152a
Results:	No Data
Reference:	Meylan, W. M. and P. H. Howard (1999). User's Guide for the ECOSAR Class Program, Version 0.993 (Mar 99), prepared for J. Vincent Nabholz and Gordon Cas, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC, prepared by Syracuse Research Corp., Environmental Science Center, Syracuse, NY 132 10 (submitted for publication).
Reliability:	Estimated value based on accepted model.

Additional References for Acute Toxicity to Fish: None Found.

4.2 Acute Toxicity to Invertebrates:

Type:	**4%hour EC_{50}**
Species:	Daphnid
Value:	**720** mg/L (log_{10} Kow of 0.75), 150 mg/L (log_{10} Kow of 1.5)
Method:	Modeled
GLP:	Not Applicable
Test Substance:	HFC-152a
Results:	No Data
Reference:	Meylan, W. M. and P. H. Howard (1999). User's Guide for the ECOSAR Class Program, Version 0.993 (Mar 99), prepared for J. Vincent Nabholz and Gordon Cas, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC, prepared by Syracuse Research Corp., Environmental Science Center, Syracuse, NY 13210 (submitted for publication).
Reliability:	Estimated value based on accepted model.

Additional References for Acute Toxicity to Invertebrates: None Found.

4.3 Acute Toxicity to Aquatic Plants:

Type:	**96-hour EC_{50}**
Species:	Algae
Value:	419 mg/L (log_{10} Kow of 0.75), 91 mg/L (log_{10} Kow of 1.5)
Method:	Modeled
GLP:	Not Applicable
Test Substance:	HFC-152a
Results:	No Data
Reference:	Meylan, W. M. and P. H. Howard (1999). User's Guide for the ECOSAR Class Program, Version 0.993 (Mar 99), prepared for J. Vincent Nabholz and Gordon Cas, U.S. Environmental Protection Agency, Office of Pollution Prevention and Toxics, Washington, DC, prepared by Syracuse Research Corp., Environmental Science Center, Syracuse, NY 132 10 (submitted for publication).
Reliability:	Estimated value based on accepted model.

Additional References for Acute Toxicity to Aquatic Plants: None Found.

5.0 Mammalian Toxicity

5.1 Acute Toxicity

Type:	**Oral Approximate Lethal Dose (ALD)**
Species/Strain:	Male rats/Crl:CD®BR
Value:	> 1500 mg/kg
Method:	Food and water were available to the rats *ad libitum*. Rats were approximately 7 weeks old upon arrival. HFC-152a was dissolved in corn oil (23 or 46 mg/mL) and kept under pressure in aerosol cans that were maintained in an ice bath. The cans were fitted with a septum from which the dosing solution was withdrawn. One rat per dose group was administered the compound by gavage. Dose levels were ***200, 300,450, 670,*** 1000, or 1500 mg/kg. Doses of 450 mg/kg and above were administered in 2 portions about 1.5 minutes apart. A dose of 1500 mg/kg was the maximum feasible dose. Rats were observed for mortality, clinical signs, and body weight over a 14-day period.
GLP:	Yes
Test Substance:	***HFC-*** 152a, purity 99.9%
Results:	No mortality occurred at any dose level. Immediately after dosing, abdominal distention related to gas evolution was evident in all rats. Lethargy was observed at 1000 and 1500 mg/kg. High carriage, wet and yellow stained

perineum, and diarrhea were observed in all rats 1 to 2 days post-dosing. No other toxicologically significant effects occurred.

Reference: DuPont Co. (1990). Unpublished Data, Haskell Laboratory Report No. 524-90 (also cited in TSCA fiche OTS0530083).

Reliability: Low because confounding factors (the use of a gas in an oral dosing suspension) interfered with test results.

Additional References for Acute Oral Toxicity: None Found.

Type: **Inhalation Approximate Lethal Concentration (ALC)**

Species/Strain: Male rats/ChR-CD®

Exposure Time: 4 hours

Value: 383,000 ppm

Method: Groups of 6 rats were exposed whole-body to 66,400, 175,200, 319,000, 383,000, and 437,500 ppm. The age of the rats was not specified, however, the initial body weight of the rats was 240 297 grams. The gas was regulated through a calibrated flowmeter into a mixing chamber. Regulated flows of air and/or oxygen were used as the carrier gas from the mixing chamber to the exposure chamber. At the 66,400 ppm level, air was used as the carrier gas. At 175,200 ppm, chamber concentrations were 16-17%. Oxygen level was maintained at about 20% for the exposure concentrations at or above 3 19,000 ppm. Chamber atmospheres were sampled at 30-minute intervals and analyzed by thermal conductivity gas chromatography. Clinical observations were recorded during the exposure and post-exposure. Gross pathology was performed on surviving rats after a 14-day observation period.

GLP: No

Test Substance: HFC- 152a, purity >99.9%

Results: Mortality ratio of 1/6 occurred at 383,000 ppm and 2/6 occurred at 437,500 ppm. Labored breathing, lethargy, and unresponsiveness to sound were observed during exposure. No clinical signs were noted following exposure. No compound-related gross pathology changes were observed.

Reference: DuPont Co. (1975). Unpublished Data, Haskell Laboratory Report No. 699-75.

Reliability: High because a scientifically defensible or guidelined method was used.

Additional References for Acute Inhalation Toxicity:

Data from these additional sources support the study results summarized above. The studies were not chosen for detailed summarization because the data were

not substantially additive to the database.

DuPont Co. (1952). Unpublished Data, Haskell Laboratory Report No. 2-52.

Izmerov, N. F. et al. (1982). Toxicometric Parameters of Industrial Toxic Chemicals Under Single Exposure, p. 54.

Carpenter, C. P. et al. (1949). J. Ind. Hyg. Toxicol., 3 1:343-346.

Lester, D. and L. A. Greenberg (1950). Arch. Ind. Hyg. Occup. Med., 2:335-344.

DuPont Co. (n.d.). Unpublished Data, MR-894-4.

Van Poznak, A. and J. F. Artusio, Jr. (1960). Toxicol. Appl. Pharmacol., 2:363-373.

Type: **Dermal Toxicity:** No Data.

Type: **Dermal Irritation:** No Data.

Type: **Dermal Sensitization:** No Data.

Type: **Eye Irritation:** No Data.

Type: **Cardiac Sensitization**
Species/Strain: Male dogs/Beagle
Value: Cardiac sensitizer at 150,000 ppm
Method: Beagle dogs (I 2/group) were exposed to 50,000 or 150,000 ppm for 5 minutes. The age of the dogs was not specified. The dogs received a control injection of epinephrine (0.008 mg/kg) intravenously, prior to exposure and a challenge injection (same dosage) after breathing the test material for 5 minutes. The desired concentrations (calculated) were achieved by delivering a metered volume of the vapor or gas from the pressured cylinder containing the test substance and diluting it with a known volume of air. The flow meter used for monitoring the test compound had been previously calibrated with the compound by a dry gas test meter.

The dogs were trained to maintain a standing position while lightly supported by a cloth sling with a hole for each leg, to wear a mask over their snout, and to accept a venupunctnre. An electrocardiogram was recorded continuously during the experimental procedure.

GLP:	No
Test Substance:	HFC-152a, purity 99.99%
Results:	Marked responses (a cardiac arrhythmia which was considered to pose a serious threat to life) were observed in 3 of the 12 dogs at the 150,000 ppm level. No response was seen at the 50,000 ppm level.
Reference:	DuPont Co. (1969). Unpublished Data, Haskell Laboratory Report No. 354-69. Reinhardt, C. F. et al. (1971). Arch. Environ. Health, 22:265-279.
Reliability:	Medium because a suboptimal study design was used. Animals were not individually titrated with epinephrine.

Additional References for Cardiac Sensitization:

Data from these additional sources were not summarized because the study designs were not adequate. The studies used anesthetized animals and/or species other than the dog.

Aviado, D. M. and M. A. Belej (1974). Toxicology, 2:3 l-42.

Brody, R. S. et al. (1974). Toxicology, 2:173-184.

Doherty, R. E. and D. M. Aviado (1975). Toxicology, 3:213-224.

5.2 Repeated Dose Toxicity

Type:	**2-Year Inhalation**
Species/Strain:	Rats/Crl:CD®BR
Sex/Number:	Male and Female020 per exposure group
Exposure Period:	*2* years
Frequency of Treatment:	6 hours/day, 5 days/week (excluding holidays)
Exposure Levels:	0, 2000, 10,000, 25,000 ppm
Method:	Food and water were available to the rats *ad libitum* except during exposures. Rats (54 days of age at the time of the first exposure) were exposed whole-body to the vapor. During exposures, chamber temperature and relative humidity were maintained at approximately 24 ± 5°C and 50 ± 10%, respectively. Chamber atmospheres were generated by metering HFC- I 52a vapors from 1 -ton cylinders of liquid HFC-152a, maintained at room temperature, through rotometers into the chamber air flow.

The control chamber received dilution air only. Chamber atmospheres were quantitatively analyzed for HFC-152a by gas chromotography.

Body weights were recorded twice monthly for the first 14 weeks, and then once monthly for the remainder of the study. All animals were observed for abnormal behavior and clinical signs of toxicity twice daily during the work week. On weekends and holidays, all cages were observed daily for animals that had died.

Ten rats/sex/group were subjected to clinical pathology evaluation at 1, 3, 6, 12, 18, and 24 months on test. Twelve hematological and 8 clinical chemistry parameters were measured or calculated. On the day prior to each bleeding time, an overnight urine specimen was collected and 12 urine parameters were measured or calculated.

Ten rats/sex/group were sacrificed and necropsied at 3 and 12 months. All surviving rats at 24 months were sacrificed and necropsied. Gross examinations were conducted on all rats and 38 tissues were saved for microscopic examination. Organ weights were recorded for 10 of the organs. Histopathological examinations were conducted on the control and high-exposure groups and on any animals that died or were sacrificed *in extremis*. In addition, kidneys and nasal tissues at the 3-month and 24-month final sacrifices, respectively, from all intermediate- and low-exposure rats received histological evaluation.

GLP: Yes

Test Substance: HFC-152a, purity >99.9%

Results: Overall means and standard deviations of the weekly average exposure concentrations were 2000 ± 100, 10,000 ± 500, and 25,000 ± 600 ppm HFC-152a.

Mean body weights and body weight gains of male and female rats were comparable or superior to their respective controls. Ocular/nasal discharges (25,000 ppm males and females), wet/stained perinea (25,000 ppm males and females), and stained body/face (25,000 females) were observed. Dose-response trends were apparent for the latter two signs. Swollen ears were significantly elevated in the 2000 ppm male and the 25,000 ppm males and females with a dose-related trend apparent in the females.

At the time of final sacrifice, mortality ratios of male rats

were 48/100, 51/100, 49/100, and 51/100 in the 0, 2000, 10,000 and 25,000 ppm groups, respectively. Mortality ratios in female rats were 54/100, 61/100, 47/100, and 471100 in the 0, 2000, 10,000 and 25,000 ppm groups, respectively.

Over the duration of the study, female rats exhibited increased mean corpuscular volumes (10,000 and 25,000 ppm) and increased serum bilirubin (all treatment groups), while male rats exhibited increased hematocrits (10,000 and 25,000 ppm), increased mean corpuscular volumes (10,000 and 25,000 ppm), and increased urobilinogin (25,000 ppm). In the absence of any abnormalities in hematopoietic tissues or red blood cell counts among either males or females or of changes in serum bilirubin in males, the above observations, which would be consistent with a hemolytic effect. provide inconclusive evidence of such a condition. While significantly lower than control values for all treated female groups when analyzed as relative numbers, eosinophils were significantly low only for the 10,000 ppm female group when analyzed as absolute numbers. Monocytes were significantly lower than control values for all treated male groups when analyzed as either relative or absolute numbers. The depression in monocytes, however, is of unknown clinical or biological significance.

A dose-related increase in urinary fluoride concentration and excretion was observed in the 10,000 and 25,000 ppm male and female rats and in the 2000 ppm males when evaluated over the entire study.

Serum creatinine was significantly elevated in both the 10,000 and 25,000 ppm female groups. The latter also exhibited increased urine volume and a decrease in urine osmolality.

At the 3-month sacrifice, differences in the following organ weights were noted: increased absolute heart, stomach, and adrenal weights (2000 ppm males); decreased absolute liver and spleen weights (25,000 ppm males); decreased relative liver and spleen weights (10,000 and 25,000 ppm males); decreased relative lung weights (2000 and 25,000 ppm males); decreased relative testes weights (10,000 ppm males); decreased relative pituitary weights (2000 ppm males); increased absolute stomach weights (2000 ppm females); increased adrenal weights (2000 and 10,000 ppm

females); decreased absolute brain weights (25,000 females); decreased relative brain weights (2000 and 25,000 ppm females); decreased absolute heart weight (25,000 females); decreased relative heart weights (all treated female groups); decreased absolute lung weights (1000 and 25,000 ppm females); decreased absolute spleen weights (25,000 ppm females); decreased relative spleen weights (all treated females); increased absolute pituitary weights (all treated females); increased relative pituitary weights (10,000 and 25,000 ppm females); and decreased relative liver weights (25,000 ppm females). Renal tubular changes were noted in females at the 3-month sacrifice. The changes consisted of slight cytoplasmic vacuolation, luminal dilation, and presence of occasional vesiculated nuclei in 4/10 males and 7/10 females in the 25,000 ppm group. Similar lesions were not seen in rats from the 2000 or 10,000 ppm groups. A pathological peer review of the study by Pathology Associates revealed no distinct evidence of HFC-I 52a-induced toxicity or carcinogenicity in any tissue examined. Preparation of additional kidney sections indicated that renal tubular changes recorded by the original pathologist in female rats sacrificed after 3 months exposure were the result of tissue processing artifact rather than treatment-related nephrotoxicity.

At the 12-month sacrifice, no differences in macroscopic lesions were detected between the treated and control groups. No compound-related neoplastic or non-neoplastic lesions were observed. Differences in mean absolute and relative organ weights of animals sacrificed at 12 months consisted of: increased absolute lung weights (10,000 ppm males); decreased absolute thymus weights (2000 ppm males); decreased relative thymus weights (2000 and 10,000 ppm males); increased relative heart and stomach weights (25,000 ppm males); decreased absolute pituitary weights (2000 and 10,000 ppm females); decreased reiative pituitary weights (all treated females); and decreased relative liver weights (25,000 ppm females).

No statistically significant difference in mean absolute or relative organ weights were noted between treated and control male rats sacrificed at 24 months. However, treated female rats presented the following differences from control in organ weights: increased absolute and relative lung weights (2000 and 10,000 ppm), increased absolute stomach weight (25,000 ppm), increased relative stomach weight (all

treated females), increased relative heart weight (2000 ppm), and increased relative liver weight (25,000 ppm).

Atrophy of the nasal olfactory epithelium was noted at the 2-year sacrifice in some rats from all but the intermediate-exposure females. Incidences of the lesions are provided in the table below. The incidence of focal mucosa! metaplasia in all treated groups were clearly not dose-related. The increased incidence of mucosal atrophy was statistically significant in the 2000 and 25,000 ppm male and female groups, although an analysis for dose-related trend was significant only in the males.

Males

Exposure (ppm)	0	2000	10.000	125,000
No. of nasal tissues examined	97	87	92	89
Focal mucosal metaplasia	57	21	21	21
Mucosal atrophy	0	5	2	8

Females

Exposure (ppm)	lo	2000	10,000	125,000
No. of nasal tissues examined	95	88	91	95
Focal mucosal metaplasia	22	17	6	23
Mucosal atrophy	0	9	0	17

Atrophy of the olfactory epithelium was confirmed by the peer review to be present in small numbers of females exposed for 2 years. These nasal changes, however, usually were unilateral in distribution and were entirely consistent with olfactory lesions, which have been described as spontaneous alterations in aging rats. Further, group incidence trends for olfactory atrophy were inconsistent with

exposure concentration gradients. Olfactory changes, therefore, were not regarded as treatment related.

A nasal adenoma in 1 male and 1 female from the 25,000 ppm groups and osteomas originating from the skull in 3 males from the 25,000 ppm group were observed at the final sacrifice. These tumor types did not occur in the concurrent controls. The incidence of neither tumor was statistically significant and each was considered to be of unclear biological significance.

FC-152a was not carcinogenic and produced no life-shortening toxic effects.

Reference: DuPont Co. (1982). Unpublished Data, Haskell Laboratory Report No. 8-82 (also cited in TSCA fiche OTS0520846).

DuPont Co. (1992). Pathology Associates, Inc., May 19, 1992.

Reliability: High because a scientifically defensible or guidelined method was used.

Additional References for Repeated Dose Toxicity:

Data from these additional sources support the study results summarized above. The studies were not chosen for detailed summarization because the data were not substantially additive to the database.

DuPont Co. (1976). Unpublished Data, Haskell Laboratory Report No. 158-76.

Lester, D. and L. A. Greenberg (1950). Arch. Ind. Hyg. Occup. Med., 2:335-344.

5.3 Developmental Toxicity

Species/Strain:	Rat/Charles River CD@
Sex/Number:	Female/27 per exposure group
Route of Administration:	Inhalation
Exposure Period:	Days 6- 15 of gestation; Cesarean section on Day 21
Frequency of Treatment:	6 hours/day
Exposure Levels:	0, 5000, 50,000 ppm
Method:	The rats were bred by the supplier (Charles River). The

morning when sperm were found in the vaginal smear was counted as Day 1 of gestation. Animals (young adults weighing approximately 185 grams) were delivered at either 4 or 2 days pregnant. Food and water were available *ad libitum* except during inhalation exposures.

Desired exposure concentrations were generated by metering the vapors of HFC-152a from the cylinder into 1.4 m^3 stainless steel and glass chambers operating under dynamic airflow conditions. Chamber atmospheres were monitored every 30 minutes via gas chromatograph (GC). The GC was calibrated daily with gaseous standards. Control animals were exposed to room air in identical chambers. Animals were observed daily for signs of toxicity and weighed periodically throughout the study. Dams were euthanized on Day 2 1 and organs of the thoracic and abdominal cavities were examined and the uterine weight was recorded. Corpora lutea, implantation sites, live and dead fetuses, resorptions, fetal weight, crown-rump length of live fetuses, and a gross external fetal examination were recorded. Half of the fetuses from each litter were examined for skeletal abnormalities. The remaining fetuses were examined for visceral and neural anomalies (via the Wilson method with modification described by Barrow and Taylor).

GLP: No

Test Substance: HFC-I 52a, purity >99.9%

Results: The daily average analytical exposure levels during the entire 10-day exposure period were 5300 ± 1200 and 45,300 ± 4900 ppm.

No compound-related clinical signs of maternal toxicity or body weight changes were observed. No gross pathological abnormalities were observed in ovaries, uterine horns, vital organs, or tissues of treated animals.

Pregnancy ratios were 22127, 21/27, and 19/27 at 0, 5000, and 50,000 ppm, respectively. A summary of other reproductive outcomes (means/litter) are provided in the table below:

Concentration (ppm)	0	5000	50,000
Corpora lutea:	11.6	11.3	12.2
Implantations:	10.0	9.4	10.5
No. of Resorptions:	1.5	1.4	1.2
Total No. of Fetuses:	9.3	8.8	9.9
Total No. of Live Fetuses:	9.3	8.8	9.9
Mean Fetal Weight (g):	4.3	4.4	4.3
Sex Ratio:	NR	NR	NR

NR = Fetal sex was not recorded; therefore, sex ratios could not be calculated.

The number of corpora lutea, implantation sites, and live fetuses per litter were similar in all groups. The post-implantation death of fertilized ova in exposed females indicated by early and late resorptions and dead fetuses was not different from that of the control group. Fetal body measurements, i.e. mean weight and crown-rump length in groups were not different from controls.

The HFC-152a treatment did not affect embryonal development as measured by gross external, visceral, and skeletal examinations. Petechial hemorrhages and small subcutaneous hematomas on various parts of the body and the number of runts among litters and fetuses were similar in all groups. Apparent hydronephrosis, transposition of the viscera, liver peliosis, and internal hemorrhage were detected; however, none of these findings were considered to be treatment-related. All skeletal changes were minor anomalies and variants and were about equally distributed in all groups.

A summary of gross, soft tissue, and skeletal anomalies are provided in the table below. Data are presented as number of litters (fetuses) affected.

Concentration (ppm)	0	5000	50,000
Gross External, Number examined:	22(205)	20(184)	19(188)
Petechial hemorrhages	4(4)	5(5)	3(5)
Hematoma	10(12)	6(7)	8(14)
Runts	2(2)	1(1)	1(1)
Soft Tissue, Number examined:	22(105)	20(93)	19(90)
Hydronephrosis (apparent)	1(1)	3(4)	2(2)
Situs inversus	0	0	1(1)
Liver peliosis	0	0	3(3)
Internal hemorrhage	1(1)	0	0
Skeletal, Number examined:	22(100)	20(91)	19(98)
14"" rudimentary rib	16(51)	16(53)	19(69)
Wavy ribs	8(17)	6(8)	5(7)
Sternebrae unossified	10(14)	9(16)	12(23)
Bipartite centra	3(3)	**0**	2(2)
Hyoid unossified	2(3)	**0**	4(6)

The NOEL for maternal and developmental toxicity was 50,000 ppm, the highest level tested.

Reference: DuPont Co. (1979). Unpublished Data, Haskell Laboratory Report No. 437-79.

Reliability: High because a scientifically defensible or guidelined method was used.

Additional References for Developmental Toxicity: None Found.

5.4 Reproductive Toxicity

Species/Strain: Rats/Crl:CD®BR

Sex/Number: Male and Female/l20 per exposure group

Route of Administration: Inhalation

Exposure Period: **2** years

Frequency of Treatment: 6 hours/day, 5 days/week (excluding holidays)

Exposure Levels: 0, 2000, 10,000, 25,000 ppm

Method: A 2-year inhalation study was conducted in male and female

	rats (see section 5.2 for details on the study design). Terminal sacrifices occurred at 3, 12, and 24 months. Ten rats/sex/group were sacrificed and necropsied at 3 and 12 months. All surviving rats at 24 months were sacrificed and necropsied. Gross examinations were conducted on all rats. Microscopic examinations were conducted on the control and high-exposure groups and on any animals that died or were sacrificed *in extremis*. Reproductive organs included in the histopathological evaluation included testis, epididymis, prostate, ovary, uterus, and vagina. The testis was weighed and mean organ to final body weight ratios were calculated.
GLP:	Yes
Test Substance:	HFC-152a, purity >99.9%
Results:	No histopathological or weight effects on reproductive organs of either male or female rats were observed. Results on other organ systems are summarized in Section 5.2.
Reference:	DuPont Co. (1982). Unpublished Data, Haskell Laboratory Report No. 8-82 (also cited in TSCA Fiche OTS0520846).
Reliability:	Medium because a suboptimal study design was used.

Additional References for Reproductive Toxicity: None Found.

5.5 Genetic Toxicity

Type:	**In vitro Bacterial Reverse Mutation Test**
Tester Strains:	*Salmonella typhimurium* strains TA97a, TA98, TA100 and TA1535 and *Escherichia coli* strain WP2*uvr*A (pKM101).
Exogenous Metabolic Activation:	With and without Aroclor®-induced rat liver S-9
Exposure Concentrations:	0, 20, 30, 40, 50, 75%
Method:	This study followed the following test guidelines: U.S. EPA Health Effects Test Guidelines OPPTS 799.95 10 (1989) OECD Guidelines for Testing of Chemicals Section 4: Health Effects, No. 471 (Adopted 1997) Commission Directive 92/69/EEC, EEC Method B. 12 The study consisted of 2 independent trials with and without a metabolic activation system. Three replicates were plated for each tester strain, test concentration, and condition.

Positive and negative controls were included in all assays. The reaction mixture (S-9 mix) contained glucose 6-phosphate, NADP, NaH_2PO_4, KCL, $MgCL_2$, distilled water, and S-9. Treatments with activation were conducted by adding 0.5 mL of S-9 mix, and 0.1 mL of an overnight culture to 2 mL of top agar. These components were briefly mixed and poured onto a minimal glucose agar plate. Treatments in the absence of the metabolic activation system were identical to those with activation with the exception that 0.5 mL of sterile buffer was used as a replacement for the S-9.

Plates were exposed to dilutions of the test gas in 6-L glass chambers. The test substance and filtered air flows were regulated using individual rotameters, and mixed prior to entry into the chambers. Chambers were placed into an incubator at 37°C for approximately 48 hours. Gas chromatographic analysis was used to confirm the concentration of test atmospheres.

Bacterial background lawns were evaluated for evidence of test substance toxicity and precipitation. Revertant colonies for a given tester strain and condition were counted by an automated colony counter.

Positive control substances tested in this study included 2-nitrofluorene, N-ethyl-N-nitro-N-nitroguanidine, sodium azide, ICR 19 1 acridine mutagen, 9, 10-dimethyl-1,2-benzanthracene, and 2-aminoanthracene.

Filtered house-line air was the test substance diluent and negative control.

A test substance was classified as positive if the mean number of revertants in any strain (except *S. typhimurium* TA1535) at any concentration was at least 2 times greater than the mean number of revertants of the concurrent negative control, and there was a concentration-related increase in the mean number of revertants per plate in that same strain. For *S. typhimurium* TA1535, there must be no test substance concentration with a mean number of revertants that is at least 3 times greater than the mean number of revertants of its concurrent negative control and a concentration-related increase in the mean number of revertants per plate. A test substance was classified as negative if all positive classification criteria for all strains

	were not met.
GLP:	Yes
Test Substance:	HFC- 152a, purity 99.99%
Results:	Negative
Remarks:	The actual concentrations for the first trial were 0, 20.4, 33 .5, 42.0, 55.5, and 82.7% in the absence of S-9 and 0, 21.7, 33.4, 43.6, 56.1, and 84.4% in the presence of S-9. No test substance-related precipitate was observed at any concentration level. Test substance-related toxicity, evidenced by the concentration-dependent reduction in the mean number of revertants per plate, was observed in the first trial in *S. typhimurium* strains TA97a (-S-9) and TA1535 (+S-9), and in the *E. coli* strain (+S-9, -S-9). The actual concentrations for the second trial were 0, 20.5, 30.9, 41.4, 53.9, and 85.1% in the absence of S-9 and 0, 20.8, 31.7, 41.9, 55.3, and 81.4% in the presence of S-9. Test substance-related toxicity, evidenced by the concentration-dependent reduction in the mean number of revertants per plate, was observed in the first second in *S. typhimurium* strains TA97a (-S-9) and TA98 (+S-9, -S-9), and in the *E. coli* strain (+S-9, -S-9). All acceptability criteria were met in this test. All tester strains exhibited appropriate phenotypic characteristics. No test substance-related precipitate was observed. The mean number of revertants in the negative control for each strain was within the prescribed acceptable historical control range. Mean positive control values for the tester strains exhibited greater than a 3-fold increase over the means of the respective negative controls in both trials. Differences between targeted and actual doses in both analyses were acceptable for the purposes of this assay and in no way impacted the integrity or validity of this study.
Reference:	DuPont Co. (2000). Unpublished Data, Haskell Laboratory Report No. DuPont-4032.
Reliability:	High because a scientifically defensible or guidelined method was used.

Additional References for *in vitro* Bacterial Reverse Mutation Studies:

Data from these additional sources support the study results summarized above. These studies were not chosen for detailed summarization because the data were not substantially additive to the database.

Araki, A. et al. (1994). Mutat. Res., 307(1):335-344.

DuPont Co. (I 977). Unpublished Data, Haskell Laboratory Report No. 73 l-77.

Longstaff, E. et al. (1984). Toxicol. Appl. Pharmacol., 72: 15-3 1.

Type:	**In *vitro* Chromosome Aberration Test**
Cell type:	Human lymphocytes
Exogenous Metabolic Activation:	With and without Aroclor®-induced rat liver S-9
Exposure Concentrations:	Test 1 (3-hour exposure with and without S-9): 0, 35, 50, 70% Test 2 (3-hour exposure with S-9): 0, 35, 50, 70% Test 2 (19-hour exposure without S-9): 0, 35, 50, 70% Test 3 (19-hour exposure without S-9): 0, 50, 60, 70%
Method:	This study followed the following test guidelines: U.S. EPA Health Effects Test Guidelines OPPTS 870.5375 (1998) OECD Guidelines for Testing of Chemicals Section 4: Health Effects, No. 473 (Adopted 1997) Human lymphocytes, in whole blood culture, were stimulated to divide by addition of phytohaemagglutinin, and duplicate cultures were exposed to the test substance. Treatment atmospheres of the test substance were prepared in sterile glass bottles with septum caps. Negative and positive control cultures were also prepared. Mitomycin C and cyclophosphamide were used as positive control substances. Air was used as the negative control substance. The test substance was sampled from the cylinder into a gas-sampling bag. Air was withdrawn from each pre-warmed (37°C) bottle and then an appropriate volume of test substance gas was introduced from the sampling bag, inserted through the septum cap, and the atmosphere was equilibrated at 37°C. After injection of the lymphocyte culture, air was allowed to enter each bottle through a hollow needle to produce the required concentration at atmospheric pressure. After approximately 48 hours, the cultures in duplicate were injected into the sterile glass bottles. The culture bottles were incubated on their sides at 37°C in a roller apparatus which rotated the bottles once every 8 minutes. Test 1 included a 3-hour treatment with and without S-9 mix

and 16 hours of recovery. Test 2 included a 3-hour treatment with S-9 mix and 16 hours of recovery, and a 19-hour continuous treatment without S-9.

Following the results of the second test, a third test was performed for a continuous treatment time, in the absence of S-9 only, to demonstrate reproducibility.

Two hours before the end of the incubation period, cell division was arrested using Colcemid®, the cells harvested and slides prepared, so that metaphase cells could be examined for numerical (polyploidy) and structural chromosomal damage.

In order to assess the toxicity to cultured lymphocytes, the mitotic index was calculated for all cultures treated with the test substance and the negative control. The highest dose level scored for chromosomal damage was, whenever possible, selected as the dose level causing a relative depression in mitotic index of at least 50%.

The test substance was considered to cause a positive response if the following conditions were met:

Statistically significant increases ($p<0.01$) in the frequency of metaphases with aberrant chromosomes (excluding gaps) were observed at one or more test concentration.

The increases exceeded the negative control range of this laboratory, taken at the 99% confidence limit.

The increases were reproducible between replicate cultures.

The increases were not associated with large changes in osmolality of the treatment medium or extreme toxicity.

Evidence of a dose-relationship was considered to support the conclusion.

A negative response was claimed if no statistically significant increases in the number of aberrant cells above concurrent control frequencies were observed, at any dose level.

GLP: Yes
Test Substance: HFC-152a, purity 99.99%
Results: Weakly positive
Remarks: No substantial toxicity (250% mitotic inhibition) was

observed at any dose level under any testing condition.

In the first test, after 3-hour exposure in the absence or presence of S-9, the test substance caused no biologically relevant statistically significant increases in the proportion of cells with chromosomal aberrations at any dose level.

In the second test, after 19-hour exposure in the absence of S-9, the test substance caused statistically significant increases in the proportion of metaphase figures containing chromosomal aberrations at the 70% dose levels both including and excluding gap-type aberrations. A trend test analysis also recorded a statistically significant dose-response. The observed increased chromosome aberration frequencies were outside the upper 99% confidence limits of the historical control range. In the presence of S-9, the test substance caused no statistically significant increase in the proportion of metaphase figures containing chromosomal aberrations at any dose level.

In the third test, after a confirmatory 19-hour exposure in the absence of S-9, the test substance caused a statistically significant increase in the proportion of cells with chromosomal aberrations only at the 60% dose level, excluding gap-type aberrations only. There was, however, no recording of a statistically significant dose-response. Cultures treated with the test substance at 50 and 70% did not show any statistically significant chromosome aberration increases.

A quantitative analysis for polyploidy was made in cultures treated with the negative control and highest dose level. No increases in the proportion of polyploid cells were seen in any test.

All positive control compounds caused large, statistically significant increases in the proportion of aberrant cells, demonstrating the sensitivity of the test system and the efficacy of the S-9 mix.

It was concluded that the test substance showed statistically significant evidence of clastogenic activity in this test system only after continuous 19-hour treatment in the absence of S-9. However, the observed positive responses were weak and considered to be of marginal biological relevance.

Reference: DuPont Co. (2000). Unpublished Data, Haskell Laboratory

Report No. DuPont-40 16.

Reliability: High because a scientifically defensible or guidelined method was used.

Additional References for *in* vitro Clastogenicity Studies: None Found.

Type: ***In vivo*** **Rat Micronucleus Test**

Species/Strain: Rats/Sprague Dawley CD

Sex/Number: Male and female/5 per sex per concentration per sampling time

Route of Administration: Inhalation

Concentrations: 0, 4875, 9750, 19,500 ppm

Method: The procedures used in the test were based on the recommendations of the following guidelines:

OECD Guideline for the Testing of Chemicals. (1997) Genetic Toxicology: Mammalian Erythrocyte Micronucleus Test, Guideline 474.

EC Commission Directive 2000/32/EC Annex 4C B. 12. Mutagenicity -In *vivo* mammalian erythrocyte micronucleus test. No. L 136/50.

US EPA (1998) Health Effects Test Guidelines; OPPTS 870.5395 Mammalian erythrocyte micronucleus test. EPA *712-C-98-226.*

Animals were treated for a single 6-hour period of whole body inhalation exposure to nominal concentrations of 4875, 9750, and 19,500 ppm. The negative control group received clean air only. The positive control group was dosed, orally, by gastric intubation, with cyclophosphamide at 20 mg/kg body weight. Rats weighed between 140 and 149 grams on dispatch from the supplier. Each group of animals was kept, with sexes separate, in cages and maintained in a controlled environment. Temperatures recorded throughout the test were in the range 21±2°C. Relative humidity was recorded in the range 34-56%. Although the lower limit was outside the normal range for this species (55± IO%), no adverse effects were recorded for any animal throughout the test and was not considered to have any affect on the integrity of the study. The room was illuminated by artificial light for 12 hours per day. Animals were provided with food and tap water *ad libitum* except during the period of inhalation exposure.

Animals were exposed in whole-body exposure chambers constructed from stainless steel and glass. The internal volume of each chamber was approximately 750 litres. Air was introduced into each exposure chamber at a total rate of 150 litres per minute. The flow through each chamber was approximately 12 air changes an hour; normally sufficient to maintain oxygen concentration above 19% v/v, temperature approximately 22 (± 1) °C and relative humidity between 40-60%. The exposure chamber was maintained 1-10 mm H_2O below ambient pressure. Animals were housed singly in stainless steel mesh compartments during exposure.

The test atmosphere was produced by diluting the gaseous test substance with air. Adjustments were made to the gaseous test substance supply to each chamber during exposures in order to maintain the desired concentrations. The dilutions of gaseous test substance with air were determined during preliminary generation trials. During exposure the chamber atmosphere was sampled to determine the concentration of test vapour on at least six occasions during each exposure. The nominal concentration of test substance was calculated by recording the amount of test substance delivered to the generation system during the exposure. The usage over the six hours exposure was divided by the total airflow through the chamber. Any losses during the generation process were quantified and included in the calculation of the nominal concentration. Airflow, chamber temperature, and humidity were monitored continuously and recorded at approximately 30-minute intervals.

Following dosing, the animals were examined regularly and any observed mortality or adverse clinical signs were recorded. Five males and five females were sacrificed from the negative control and each of the test substance groups 24 hours after completion of the exposure period and from the positive control group 24 hours after dosing. In addition 5 male and 5 female animals were sacrificed from the negative control and high level treatment groups 48 hours after completion of the exposure period.

The animals were killed by cervical dislocation following carbon dioxide inhalation and both femurs dissected out from each animal. The femurs were cleared of tissue and the proximal epiphysis removed from each bone. The bone marrow of both femurs from each animal was flushed out and

pooled in a total volume of 10 mL Hanks' balanced salts solution by aspiration. The resulting cell suspensions were centrifuged at 1000 rpm (150 x g) for 5 minutes and the supernatant discarded. Each resulting cell pellet was resuspended in 2 mL of filtered foetal calf serum before being sedimented by centrifugation. The supernatant was discarded and the final cell pellet was resuspended in a small volume of foetal calf serum to facilitate smearing in the conventional manner on glass microscope slides. Several smears were prepared from each femur.

Due to the presence of mast cell granules in rat bone smears, which appear identical to micronuclei when stained using the Romanowsky methods, a modified Feulgen staining method was employed. This method specifically stains DNA-containing bodies deep purple while leaving mast cell granules unstained. The method also allows reasonable differentiation of mature and immature erythrocytes and produces permanent preparations. The stained smears were examined (under code) by light microscopy to determine the incidence of micronucleated cells per 2000 immature erythrocytes (polychromatic erythrocytes, PCE) per animal. The proportion of PCEs was assessed by examination of at least 1000 erythrocytes from each animal.

GLP: Yes

Test Substance: HFC-152a, purity 99.99%

Results: Negative

Remarks: The exposure mean concentrations of the test material were 503 1, 9413 and 19,030 ppm compared with target levels of 4875, 9750 and 19,500 ppm, respectively, expressed in terms of volume of test substance vapour per unit volume of atmosphere.

Neither mortality nor adverse clinical signs was observed in the micronucleus test for any animal in any dose group over the duration of the test.

No statistically significant increases in the frequency of micronucleated PCEs and no substantial decrease in the proportion of PCEs were observed in rats treated with the test substance and killed 24 or 48 hours, compared to negative control values.

The positive control compound, cyclophosphamide, produced large, highly significant increases in the frequency of micronucleated PCEs and a decrease in the proportion of

PCEs.

It was concluded that the test substance did not show any evidence of causing chromosome damage or bone marrow cell toxicity when administered by whole body inhalation exposure in this *in vivo* test procedure.

Reference: DuPont Co. (2001). Unpublished Data, Haskell Laboratory Report No. DuPont-5426.

Reliability: High because a scientifically defensible or guidelined method was used.

Type: ***In vivo*** **Sex-linked Recessive Lethal Test**

Species/Strain: *Drosophila melanogaster*/Canton-S

Sex/Number: Female and male/not applicable

Route of Administration: Inhalation

Concentrations: 100%; flow rate of 10 mL/min

Method: The Canton-Special (Canton-S) strain was obtained from the laboratory of Dr. Luolin Browning of Houston Texas. The cultures were kept under standard laboratory conditions in an air conditioned room at approximately 22°C. Newly hatched (< 12 hours old) unetherized virgin flies were used.

A Turner bulb apparatus was used. The gas was passed through a cotton trap and from there into a dry gassing chamber that contained the flies. From this Turner bulb, tubing connected with the inlet of a second Turner bulb, which contained 15 mL of water, into which the gas was bubbled. In this way, 100 ± 5 bubbles per minute could easily be counted. The rate of gas flow in mL/min, as determined by bubble count, was also measured by water displacement. The gassing time was 5 minutes with a flow rate of 10 mL/min, with the flies remaining in the gaseous atmosphere another 5 minutes. The treated flies were then placed in a clean container and observed. Upon recovery, the flies were lightly etherized and the males placed in 1/2 pint culture bottles. The females were discarded. Using the Basc technique, untreated Muller-5 virgin females were placed with Canton-S treated males. The Basc technique was used for scoring sex-linked recessive lethal mutations that arose in the germ line of the treated paternal male. 'The protocol for scoring semilethal (mosaic lethal) recessive mutations was to further test any F_2 culture having a ratio of at least 10 heterozygous females, or I I Basc males, to 1 normal male, and if this ratio continued in the F_3 generation, to score the culture as a semilethal mutation.

GLP:	No
Test Substance:	HFC-152a, purity >98%
Results:	Positive
Remarks:	Individual lethal tests of 276 F_1 females gave 1 lethal and 8 semi-lethal, each scored as 0.5 lethal, giving a lethal count of 5/276 chromosomes. A lethal mutation frequency of 1.5% was calculated; this value was corrected for a spontaneous frequency of 0.23%. A number of developmental type abnormalities were noted among F_2 progeny. One heterozygous female had ocelli in place of proboscis and no proboscis was present. Eye color mutants transmitted were white, apricot, and a deep orange.
Reference:	Foltz, V.C. and Fuerst, R. (1974). Environ. Res., 7(3):275:285.
Reliability:	Not assignable because limited study information was available.

Additional Reference for *in vivo* Studies:

Data from this additional source support the study results summarized above. The study was not chosen for detailed summarization because the data were not substantially additive to the database.

Garrett, S. and R. Fuerst (1974). Environ. Res., 7(3):286-293.